Posttranslational Characterization of S-Adenosylmethionine Decarboxylases by LC-MS/MS

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Objectives

- **To study the posttranslational processing of AdoMetDC by LC-MS/MS.**
- **To characterize the in vivo cysteine modification**

Introduction

S-Adenosylmethionine decarboxylase (AdoMetDC) from *Escherichia coli* is an enzyme that catalyzes the decarboxylation of S-adenosylmethionine to decarboxylated S-adenosylmethionine, which subsequently serves as a substrate for propylaminotransferases in the polyamine biosynthetic pathway (Fig. 1)[1]. AdoMetDC belongs to the small group of pyruvoyl containing enzymes and is synthesized as a proenzyme, which is autocatalytically cleaved into two subunits, an 18 kDa α subunit and a 12 kDa β subunit (Fig. 2).[1] In the unprocessed native proenzyme, six cysteines are found at positions 19, 24, 56, 115, 129, 140 (Fig. 6). Cysteines that play an important role in protein function were investigated for any post-translational modifications by digestion and LC-MS/MS.

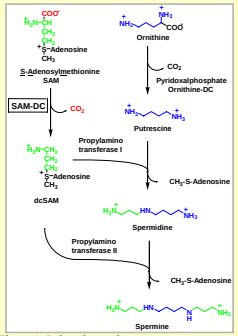

Figure 1: Polyamine pathway.

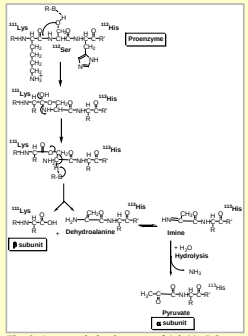

Fig. 2: Autocatalytic cleavage of AdoMetDC.

Methods

His-tagged AdoMetDC was overexpressed in *E. coli* and purified as previously described. AdoMetDCs were investigated by LC-MS. Enzymatic activity was assessed using [1-^{14}C]AdoMet. AdoMetDCs were digested with trypsin and the peptides were assayed for posttranslational modifications by LC-MS/MS using a QTOF 2 mass spectrometer. Chromatographic separation of the peptide fragments was done using a Zorbax C18SBW reversed phase column (10 cm x 0.15 μm ID) using a gradient of 0.2% formic acid and 98.8% acetonitrile containing 0.2% formic acid. Flow rate through column was 1 ul/min. Peptides were monitored by MS survey scans and MS/MS.

Conditions of LC-MS/MS:
Mobile phase:
Solvent A: 0.2% formic acid
Solvent B: ACN, 0.2% formic acid, 1% water
Sample size: < 1 pmol
Gradient: 10-90% solvent B
Flow rate: 10 μL/min, 1: 10 split

Stationary phase:
Zorbax C18 SBW column, 0.15 μm x 10 cm

Fig. 3: Instrumentation: Micromass CapLC Q-TOF2.

Results

AdoMetDC subunits were characterized by ESI-MS before and after treatment with DTT. When DTT treated subunits were compared with native subunits, a mass difference of 2 Da suggested a disulfide bond in each fragment. In addition, in substrate inactivated enzymes a +57±1 Da addition was found for the α subunit and a +76±1 Da addition was found for the α and β subunit.

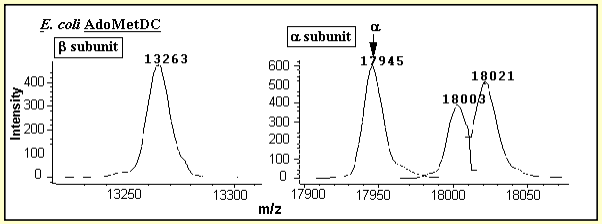

Fig. 4: LC-MS spectra of the **α**- and **β**-subunits.

To further characterize the posttranslational modifications, tryptic digestions were carried out. A sequence coverage of > 96% was achieved with a single tryptic digest. The signal at m/z 1522.74 was in excellent agreement with the expected M+ value of the disulfide containing peptide T5 and was assigned to a disulfide bond between Cys19 and Cys24. The second disulfide containing peptide (T12) was found at m/z 2350.08. The tryptic fragment T12 contained the pyruvate group of the α subunit and has a theoretical m/z 2352.04 in the reduced form and 2350.08 in the disulfide-containing form. Furthermore, an Ala analog, which is the product of a transamination reaction at the pyruvate, with an m/z 2353.01 was also observed. It was concluded that Cys115 and Cys129 form a second disulfide.

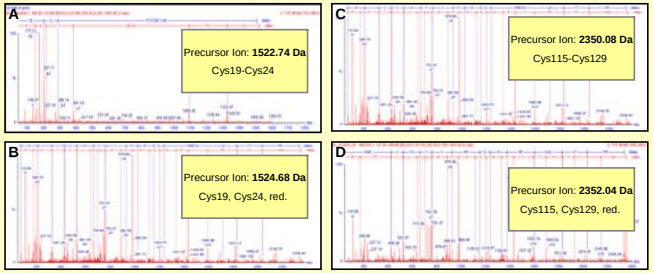

Fig. 5A-E: LC-MS/MS spectra of the disulfide containing peptides.

Fig. 6: Sequence of his-tagged AdoMetDC.

Sequence homology between species is considered low. However, all species known so far contain the TCG-motif, which is supposed to be involved in the autocatalytic processing.

For the free cysteine containing tryptic peptides T8 and T13, signals at m/z 2243.22 and 1530.80 were observed, respectively. In substrate inactivated AdoMetDC isolates that showed a considerable amount of +57 and +76 modification (Fig. 4), additional fragments were observed[2,3]. For the tryptic fragment T8 only the +76 modification was found at m/z 2319.20. For the tryptic fragment T13, an m/z 1586.60 was observed together with an m/z 1606.78 for its +76 modification (Fig. 7). The +57 addition was constituted of a +56 addition on a cysteine and a + 1 addition from the above mentioned pyruvate to Ala transamination. The +56 addition was postulated as an acrolein adduct, whereas the +76 addition was a mercaptoethanol adduct (Fig. 8).

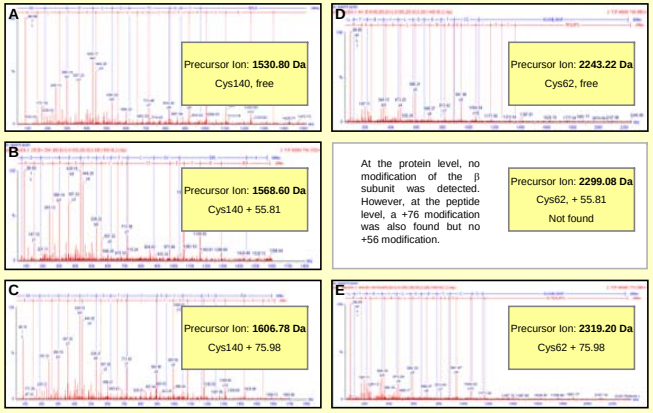

Fig. 7A-E: LC-MS/MS spectra of the peptides that contain 'free' cysteines.

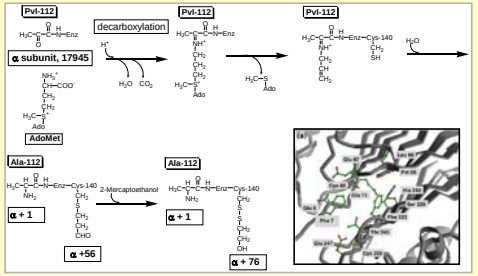

Fig. 8: Proposed mechanism for the cysteine modification *in vivo*.

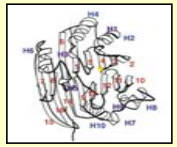

Fig. 9: Crystal structure[4].

As evident from the crystal structure of the human AdoMetDC[4], the cysteine of the TCG motif is in close proximity to the pyruvate group.

Conclusions

AdoMetDC undergoes intensive post-translational processing. Disulfide bonds are formed between C19 and C25, and between C114 and C129. Cys140 from the TCG containing motif undergoes a mechanistic acrolein addition, which inactivates the enzyme. The acrolein adduct can be replaced by a nucleophile such as mercaptoethanol.

References

1. Tabor CW, Rabor H (1984) Ann. Rev. Biochem. 53, 749-790.
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4. Ekstrom JL et al. (1999) Structure 7, 583-595.